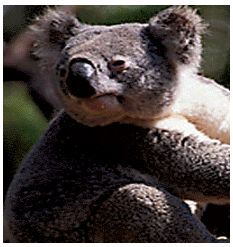
CHAPTER 20
BIOTECHNOLOGY AND RECOMBINANT DNA



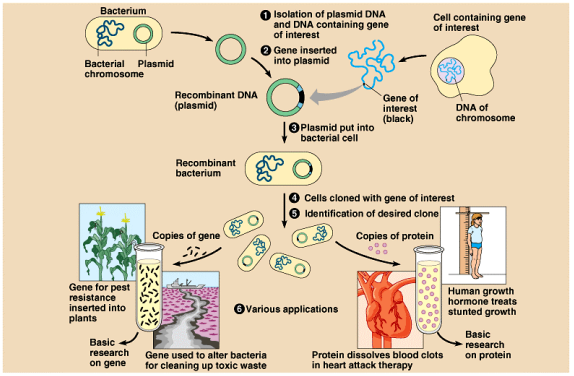
RECOMBINANT DNA TECHNOLOGY

- E.COLI, EARLY 1970'S.
- TEST TUBE: COMBINE GENES FROM DIFFERENT SOURCES.
- TRANSFER RECOMBINED FORMS OF DNA INTO CELLS.
- CELLS REPLICATE.
- THEN PROTEIN TRANSCRIPTION AND TRANSLATION= NEW PROTEIN!

DNA TECHNOLOGY

- MANIPULATING DNA.
- HUMAN GENOME PROJECT; MAPPING ALL OF HUMAN DNA/CHR.
- ENGINEERING BACTERIA TO MAKE USEFUL BYPRODUCTS; E.G. INSULIN,
- INTERFERONS, HGF, FACTOR 8 FOR BLOOD CLOTTING.

Figure 20.1 An overview of how bacterial plasmids are used to clone genes



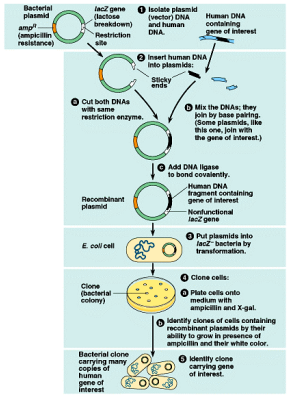
DNA TRANSFER/BACTERIA

- **TRANSFORMATION: FRAGMENT OF DNA ENTERS ANOTHER CELL AND COMBINES WITH OLD DNA.**
- **TRANSFORMS HARMLESS SPECIES TO HARMFULSPECIES/PNEUMONIA.**
- **TRANSDUCTION: VIRAL PHAGE INSERTS DNA INTO HOST CELL.**
- **CONJUGATION: TRANSFER OF DNA FROM DONOR TO RECIPIENT CELL.**
- **MAKES HYBRID RESISTANT STRAINS.**

BACTERIAL PLASMIDS

- **F FACTOR, A DNA FRAGMENT.**
- **CONJUGATION TO ANOTHER BACTERIA, TRANSFERS CHR. DNA.**
- **TRANSFER OF PLASMID, A CIRCULAR DNA . FRAGMENTS IN BACTERIA.**
- **R-PLASMIDS: DESTROY ANTIBIOTICS EFFECTIVENESS BY MAKING SPECIAL ENZYMES.**
- **PLASMID CUSTOMIZE BACTERIA.**

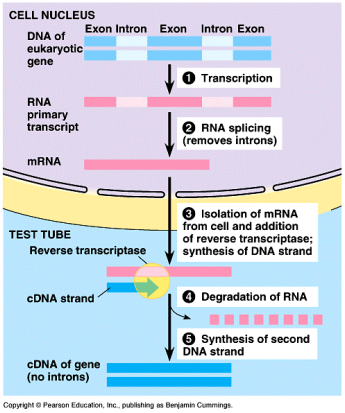
Figure 20.3 Cloning a human gene in a bacterial plasmid: a closer look (Layer 3)



USES OF BIOGENETIC TECHNOLOGY

- **GENES IN PLANTS: RESISTANT TO COLD, HEAT, DROUGHT, EXCESS WATER, VIRUSES, BACTERIA AND FUNGI.**
- **ALTERING GENES FOR HUMANS: ENZYMES HAVE A CUT AND PASTE FUNCTION, RESTRICITION ENZYMES CUT OUT UNWANTED DNA, LIGASES PASTE THE MESSAGES TOGETHER.**

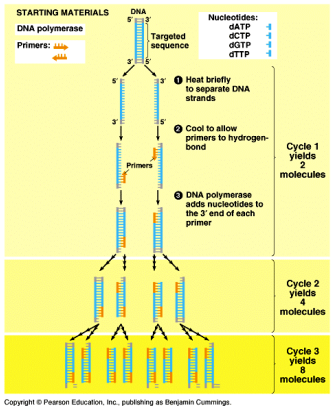
Figure 20.5 Making complementary DNA (cDNA) for a eukaryotic gene



GENOMIC LIBRARY

- RECOMBINANT DNA, MUST CLONE AND STORE RECOMBINANT .
- PLASMDS/DNA IS ALTERED AND CLONED.
- PHAGE/RECOMBINANT DNA CLONED
- MAKING EXTRA COPIES TO STORE FOR FUTURE USE BY CELL.

Figure 20.7 The polymerase chain reaction (PCR)



OTHER TOOLS FOR DNA TECHNOLOGY

- A. <u>REVERSE TRANSCRIPTASE</u>
- 1. TRANSCRIPTION IN THE CELL
- 2. RNA TRANSCRIPT (m-RNA), INTRONS ARE REMOVED, KEEP THE EXONS AFTER SPLICING.
- 3. ADD m-RNA AND REVERSE TRANSCRIPTASE, SYNTHESIZE NEW STRAND, THEN 2ND STRAND

MORE TOOLS CONTINUED:

- **B. NUCLEIC ACID PROBES**
- **. LABEL NUCLEIC ACID/DNA OR RNA, USING PROBE TECHNIQUE**
- **. RADIOACTIVE ISOTOPE COMBINES WITH DNA/RNA**
- **DEVELOP RESULTS ON FILM, GAMMA PARTICLES LEAVE RESULTS.**

Figure 20.10 Restriction fragment analysis by Southern blotting

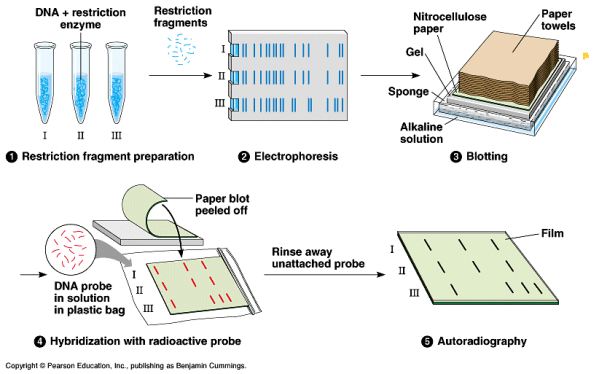
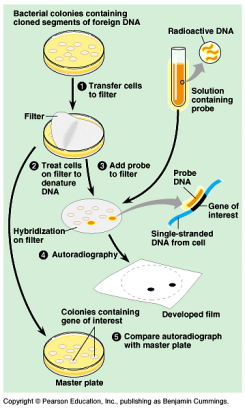


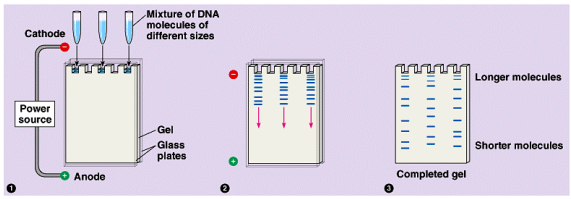
Figure 20.4 Using a nucleic acid probe to identify a cloned gene



MORE TOOLS CONTINUED:

- **C. GEL ELECTROPHORESIS:**
- **SORTS OUT DNA BY SIZE OF MOLECULES, USE OF -,+ ELECTRODE**
- **DEVELOP ON GEL AND READ.**
- **MOLECULES THAT ARE HEAVY TRAVEL SLOWLY, LIGHTER MOLECULES TRAVEL FASTER.**

Figure 20.8 Gel electrophoresis of macromolecules



MORE TOOLS CONTINUED

- **D. RESTRICTION FRAGMENT**
- **. PREPARE SAMPLE**
- **. ANALYZE RFLP, DNA SEGMENTS ARE COMPARED / AUTORADIOGRAPH.**
- **. DETECTING HARNMFUL ALLELES**
- **. SOUTHERN BLOTTING.**
- **.E. PCR METHOD:AMPLIFICATION OF DNA SEQUENCES, CLONE.**

Figure 20.13 Alternative strategies for sequencing an entire genome

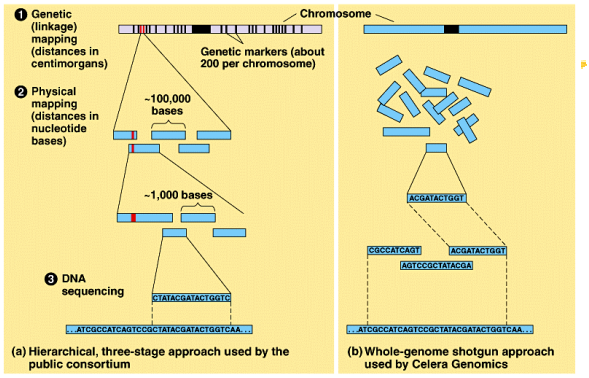


Figure 20.19 Using the Ti plasmid as a vector for genetic engineering in plants